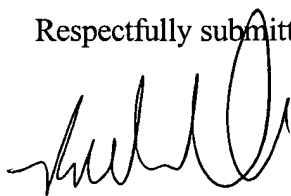


Serial No. 09/931,733  
Filed: August 17, 2001

### REMARKS

The above amendments are in response to a Notice of Incomplete Nonprovisional Application, serving to cancel all references to omitted drawings.

Respectfully submitted,



Attorney/Agent for Applicant(s)

Robert A. Silverman

(Reg. No. 35,682)

340 Kingsland Street

Nutley, New Jersey 07110

Telephone: (973) 235-2863

Telefax: (973) 235-2363

115760

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

Deleted subheading -- ~~Brief Description of the Drawings~~ --

Paragraphs 0015, 0016, 0046, 0047, 0048, and 0051 were deleted as follow:

-- ~~[0015] Figure 1 shows expression of genes encoding tumor associated antigens and IFN- $\alpha$  receptor in melanoma cell lines. Panel A shows expression of genes encoding tumor associated antigens. Panel B shows IFN- $\alpha$  receptor gene expression is detected in IFN- $\alpha$  sensitive and resistant melanoma cell lines by 25 cycles RT-PCR. --~~

-- ~~[0016] Figure 2 shows genes preferentially expressed in IFN- $\alpha$  sensitive or resistant melanoma cell lines. Panel A and Panel B show combined data sets for oligonucleotide array expression data collected from the sensitive (CNCM I-2544 (A375), CNCM I-2546 (ME15), CNCM I-2547 (ME51) and CNCM I-2548 (ME59)) or resistant cell lines (CNCM I-2545 (D10) and CNCM I-2549 (ME67)). --~~

-- ~~[0046] The examples below are in connection with the following figures:--~~

-- ~~[0047] Figure 1. Expression of genes encoding tumor associated antigens and IFN- $\alpha$  receptor in melanoma cell lines. --~~

-- ~~[0048] Panel A: Expression of genes encoding tumor associated antigens. --~~

-- ~~[0051] Figure 2. Genes preferentially expressed in IFN- $\alpha$  sensitive or resistant melanoma cell lines. --~~

Paragraphs 0049, 0050, 0052, 0065, 0068, 0072, and 0073 were amended as follows:

-- [0049] The cell lines CNCM I-2544 (375), CNCM I-2545 (D10), CNCM I-2546 (15), CNCM I-2547 (51), CNCM I-2548 (59) and CNCM I-2549 (67) were cultured for 48 hours in the presence (+) or absence (-) of 100U/ml IFN- $\alpha$ . The expression patterns of genes encoding tyrosinase, tyrosinase related protein-2, pmel-17 and mart-1, HLA restricted, tumor associated antigens ~~are~~ were reported. ~~Grey bars refer to IFN- $\alpha$  sensitive cell lines and black bars refer to IFN- $\alpha$  resistant lines (see Table 1). Data are presented as average difference of signal intensity between match and mismatch probesets.~~ --

-- [0050] ~~Panel B:~~ IFN- $\alpha$  receptor gene expression is detected in IFN- $\alpha$  sensitive and resistant melanoma cell lines by 25 cycles RT-PCR. --

-- [0052] Oligonucleotide array expression data were collected from untreated cells. Data from the sensitive (CNCM I-2544 (A375), CNCM I-2546 (ME15), CNCM I-2547 (ME51) and CNCM I-2548 (ME59)) or resistant cell lines (CNCM I-2545 (D10) and CNCM I-2549 (ME67)) were combined into two data sets ~~(panel A and panel B)~~. Average values for individual genes were then filtered to identify genes upregulated at least three fold in either group. ~~Data are presented as average difference of signal intensity between match and mismatch probesets.~~ --

--[0065] Datasets for genes encoding MART-1/Melan-A, pmel-17 (gp100), TRP-2 and tyrosinase tumor associated antigens (TAA) were first analyzed. These four genes were found to be expressed in CNCM I-2546 and CNCM I-2545 cell lines, whereas virtually no expression was detectable in CNCM I-2544, CNCM I-2546, CNCM I-2547 and CNCM I-2548 cell lines ~~(Figure 1)~~. Functional tests confirmed these findings. Indeed, CNCM I-2545, HLA-A2.1 positive melanoma cells were effectively killed by HLA-A2.1 restricted CTL lines recognizing epitopes derived from

MART-1/Melan-A, pmel-17/gp100, tyrosinase or TRP-2 proteins. In contrast, CNCM I-2548 HLA-A2.1 positive cells, that do not express the genes under investigation failed to be killed by the specific CTL (~~Figure 1~~). Remarkably, IFN- $\alpha$  treatment does not appear to influence the expression of the genes encoding these TAA. --

[0068] IFN- $\alpha$  receptor gene expression was evaluated by using a more sensitive RT-PCR assay (~~Figure 2B~~).

[0072] This analysis resulted in the identification of a group of four genes preferentially expressed in IFN- $\alpha$  sensitive cell lines (~~Figure 2, panel A~~). Two of them, IFI16 and RCC1 encode nuclear proteins endowed with mitotic regulation and transcriptional activation capacities, respectively. A third is the hox2 homeobox gene, whereas the fourth, h19 gene, encodes an untranslated RNA, involved in the DNA methylation and genetic imprinting processes. Notably, however, one of the IFN- $\alpha$  sensitive cell lines, ME51, does not express RCC1.

[0073] On the other hand, two genes encoding likely components of signal transduction pathways, SHB and PKC- $\zeta$ , appeared to be preferentially expressed in IFN- $\alpha$  resistant D10 and ME67 cell lines (~~Figure 2, panel B~~).